PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/013427	26.11.2004	09.12.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/82

Applicant
DSM IP ASSETS B.V.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/013427

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	-

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/EP2004/013427

Re Item V.

1. Reference is made to the following documents:

D1: HOLMBERG I ET AL: "DETERMINATION OF 25 HYDROXY VITAMIN D-3 IN SERUM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ISOTOPE DILUTION MASS SPECTROMETRY" SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION, vol. 44, no. 4, 1984, pages 275-282, XP009052947 ISSN: 0036-5513

D2: LUQUE DE CASTRO M D ET AL: "Determination of vitamin D3 metabolites: State-of-the-art and trends" JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, vol. 20, no. 1-2, June 1999 (1999-06), pages 1-17, XP002342738 ISSN: 0731-7085

D3: RYCHENER M ET AL: "A SIMPLIFIED AND IMPROVED DETERMINATION OF VITAMIN D IN FAT OIL AND MARGARINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY" MITTEILUNGEN AUS DEM GEBIETE DER LEBENSMITTELUNTERSUCHUNG UND HYGIENE, vol. 76, no. 1, 1985, pages 112-124, XP009052981 ISSN: 0026-6841

D4: HEUDI O ET AL: "Simultaneous quantification of Vitamins A, D3 and E in fortified infant formulae by liquid chromatography-mass spectrometry" JOURNAL OF CHROMATOGRAPHY, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 1022, no. 1-2, 2 January 2004 (2004-01-02), pages 115-123, XP004473612 ISSN: 0021-9673

2.1 Document D1 discloses (the references in parentheses applying to this document): a process for the quantitative determination of 25-hydroxy-vitamin D3 in serum comprising the steps of using directly the serum, adding tritium-labelled 25-hydroxyvitamin D3 (see scheme II), extracting with methanol/chloroform, submitting the extract to a preparative straight phase HPLC, collecting fractions and submitting them to reverse phase HPLC combined with quantification by multiple ion detection.

The differences between D1 and present claim 1 can therefore be seen in step a)

dispersing the feed sample in water and b) extracting the aqueous dispersion with tert-butyl methyl ether.

Thus, the problem solved by the present application is the provision of an alternative process for the quantification of 25-hydroxy-cholecalciferol.

D2, which is a review on the determination of vitamin D3 metabolites, discloses sample pretreatment based on (i) deproteinization steps with reagents such as acetonitrile, ammonium sulphate or ethanol or (ii) pretreatment based on saponification, which appears to be mainly used for samples containing high content of lipids such as infant formula, enriched milk, eggs, fish oil, etc followed by liquid-liquid extraction (see D2, p.6, right col. last two paragraphs and D3). D2 further comments on (iii) liquid-liquid extraction as a time-consuming method with shortcomings such as loss of analyte.

D3 teaches the sample pretreatment according to D2(ii).

D4, which was published after the priority of the present document, discloses the quantification of vitamin A, D3 and E in fortified infant formulae by liquid chromatography-mass spectrometry. The food pretreatment is according to D2(ii).

Therefore, the person skilled in the art, taking into consideration the teaching of D2 and D3, would have overcome scientific prejudice by coming up with the idea of simply dispersing the feed and thereafter extracting the analyte and internal standard with tert-butyl methyl ether.

The method of claim 1 is enabled by the examples and it has been shown that the above stated problem is solved.

Therefore, the solution provided to the above stated problem as given in claim 1 involves an inventive step.

The dependent claims 2-9 add features to the process of claim 1 and thus also relate to novel and inventive subject-matter.

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International application No.

PCT/EP2004/013427